IDENTIFICATION OF ENTEROTOXIGENIC *Escherichia coli* ISOLATED FROM SWINE WITH DIARRHEA IN THAILAND BY COLONY HYBRIDIZATION USING THREE ENTEROTOXIN GENE PROBE

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OBJECTIVE : To use the DNA colony hybridization assay to identify enterotoxigenic *Escherichia coli* among *E. coli* isolated from swine with diarrhea at 14 farms in Thailand.

BACKGROUND : Enterotoxigenic *Escherichia coli* have been implicated in diarrheal disease of swine as well as other species of animal and man (1,7-10). ETEC produce a heat-labile toxin (LT) identified by testing culture supernatants of *E. coli* in the Y-1 adrenal cell assay (6) and a heat-stable toxin (ST) identified by the suckling mouse assay (2). Both techniques are time consuming and the assay for ST requires a large number of suckling mice which are frequently not available. To determine the prevalence of ETEC as an enteric pathogen in pigs in Thailand, *E. coli* isolated from swine with diarrhea in farms at four different locations in Thailand were examined for homology with three enterotoxin gene probes (5). Colonies which were positive in the DNA colony hybridization assay were tested in the more conventional assays.

The application of this molecular genetic technology provides a method of testing a large number of *E. coli* isolated from swine for enterotoxigenicity and to further characterize genes coding for ST enterotoxins.

METHODS :

Bacterial strains : Between September 9 and December 8, 1981, 781 *E. coli* were isolated from swine (one isolate/animal) at 14 different pig farms at four different locations in Thailand. These *E. coli* were recovered from 88 litters of pigs with diarrhea between one and 21 days of age. All isolates were stored on nutrient agar slants until tested for homology with the enterotoxin gene probes. *E. coli* K12 C600 containing the following plasmids were used to prepare DNA fragments employed as enterotoxin gene probes pEWD 299 (LT), pRIT 10036 (ST-P), and pSLM 004 (ST-H). The LT probe DNA was prepared from pEWD 299, the ST-P probe DNA from pRIT 10036, and the ST-H probe from pSLM 004. Both the LT and ST-P probes were initially derived from ETEC of procine origin while the ST-H probe wad derived from an ETEC of human origin.

Preparation of α-^{32}P labelled enterotoxin gene probes : Plasmid DNA was isolated from *E. coli* K12 C600 (pEWD 299), (pRIT 10036), and (pSLM 004) and cleaved with restriction endonucleases as previously described (5). DNA fragments were isolated by polyacrylamide gel electrophoresis of digested DNA and the appropriate fragment removed by electrolution. The isolated DNA

fragment which were used as a probes for enterotoxin genes were labelled *in vitro* with α-^{32}P deoxynucleotide triphosphate (New England Nuclear, Boston, Mass.) by nick translation (5).

DNA colony hybridization assay : Each colony to be tested was inoculated onto three different 3 x 5 cm pieces of nitrocellulose paper (BA-85; Schleicher and Schuell, Kenne, NH) layered on MacConkey agar. *E. coli* K12 C600 containing the plasmids from which the enterotoxin gene probes were derived as well as LT and ST producing ETEC B2 which hybridized with the LT and ST-H probes and non-enterotoxigenic *E. coli* K12 Xac were included as controls. After incubation at 37°C for 24 hours the papers were removed from the MacConkey agar and placed onto pieces of Whatman no. 3 paper (Whatman, Clifton, NJ) saturated with 0.5 N NaOH. After ten minutes the nitrocellulose papers were transferred to similar Whatman paper saturated with 1.0 M ammonium acetate and 0.02 N NaOH for one minute. This process was repeated four times and after the last transfer the papers were kept on the saturated Whatman paper for ten minutes. The papers were then air dried and baked at 65°C overnight. Each paper was hybridized with one of the three α-^{32}P labelled enterotoxin gene probes, LT, ST-H, and ST-P. The nitrocellulose papers were then exposed to X-Omat R X-ray film (Eastman Kodak, Rochester, NY) with a single Cronex Lightening-Plus intensification screen (DuPont de Nemours, Wilmington, Del.) for 48 hours at -70°C.

E. coli (one/piglet) from 781 piglets with diarrhea were examined with the DNA colony hybridization assay. Isolates from 28 of 88 litters hybridized with the enterotoxin gene probes. All of the 49 colonies which hybridized with the LT probe produced LT as determined by the Y-1 adrenal cell assay and 68 colonies which hybridized with the ST-P enterotoxin gene probes produced ST as determined by the suckling mouse assay. No *E. coli* isolated from piglets with diarrhea hybridized with the ST-H probe derived from an ST ETEC of human origin.

As shown in Table 1, ETEC, the majority of which produced ST only, were isolated more frequently from litters of piglets under ten days of age than the older litters of animals, LT and ST and LT ETEC were uncommon in the piglets with diarrhea that were examined.

The DNA colony hybridization assay was used to determine the incidence of ETEC infection in swine at fourteen pig farms in Thailand. It was possible with this new technique to screen a large number of colonies for enterotoxigenicity. This methodology would be a valuable tool in further defining the importance of this enteric pathogen in swine as well as man.

ETEC have previously been isolated from animals living in close proximity to man (1,3) suggesting ETEC may be a zoonotic infection. The serotypes of ETEC isolated from animals are, however, different from serotypes of ETEC commonly isolated from man in Thailand hybridized with the ST-H rather than the ST-P enterotoxin gene probes (4). Although these observations are preliminary, these results suggest that ST ETEC which infect man are different from those which infect swine. Further studies employing the DNA hybridization technique are in progress in a rural community in Thailand to determine the role of livestock in the epidemiology of ETEC infections in man.

Table 1. Enterotoxigenic *Escherichia coli* infections in 88 litters of swine with diarrhea at 14 farms in Thailand in 1981.

Farm #	Date	Location	Total # of pigs	Median #/litter	# litters with ETEC	Type of ETEC infection
			<10 days of age			
SW050	Sept. 5	Sri Racha	133	8	2/17	2 ST
SW044	Sept. 5	Nakorn Pathom	12	4	3/3	3 ST
SW045	Sept. 5	Nakorn Pathom	5	3	2/2	2 ST
SW046	Sept. 5	Nakorn Pathom	25	6	4/4	4 ST
SW047	Sept. 5	Nakorn Pathom	2	1	2/2	2 ST
SW055	Sept. 17	Sri Racha	144	10	6/15	1 LTST
						2 LT, 3 ST
SW085	Nov. 27	Panat Nikom	103	7	2/4	2 ST
SW088	Dec. 8	Nakorn Pathom	95	9	2/11	2 LT
			10-21 days of age			
SW060	Sept. 26	Panat Nikom	64	8	1/8	1 ST
SW065	Oct. 2	Rayong	72	12	2/6	2 ST
SW069	Oct. 14	Panat Nikom	37	5	0/7	-
SW081	Nov. 14	Rayong	29	10	0/3	-
SW083	Nov. 14	Panat Nikom	50	10	2/5	1 LT ST
SW086	Dec. 8	Rayong	10	10	0/1	-
Total			781	7	28/88	4 LTST
						4 LT
						22 ST

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